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TITLE: MALARIA, LEISHMANIASIS & SHISTOSOMIASIS VECTOR ECOLOGY, TRANSMISSION, IMMUNOLOGY & PROPHYLAZIS IN KENYA

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A malaria vaccine evaluation site at Saradidi, Kenya, has been characterized for epidemiological, entomological and demographic parameters over a two year period. The transmission of flaciparum malaria by Anopheles gambiae s.l. and A. funestus was found to be intense year-round. An evaluation of a WRAIR-developed malaria sporozoite vaccine, R32ToxA, was begun in April, 1990, with 38 pairs of volunteers.

Leishmaniasis studies have described several parasite-vector-host relationships previously unreported in East Africa which may be important in the diagnosis or control of the disease. In particular, the isolation of two species of leishmania with differing drug sensitivity profiles from a single patient is notable. Collaborative development of a vector-induced model for infection in vervet monkeys has significant potential for vaccine evaluation in a non-human model.

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Mid-term Report

Grant DAMD17-89-Z-9032

MALARIA, LEISHMANIASIS, and SCHISTOSOMIASIS VECTOR ECOLOGY, TRANSMISSION, IMMUNOLOGY, and PROPHYLAXIS IN KENYA,

The research proposed in this grant has been successful in producing a number of findings significant to the relevant scientific fields. In order to keep this interim report to a reasonable length, citations and abstracts of published papers and manuscripts will be used to present research results.

INTRODUCTION:

Malaria:

In most tropical areas of the world, and particularly in Africa, malaria is a major cause of morbidity and mortality. Immunity to malaria can be induced in man and other animals by injection of live, irradiated sporozoites. This immunity is mediated by antibody and is species and stage specific. Dame et al. reported the successful cloning and sequencing of the gene that encodes the circumsporozoite (CS) protein of Plasmodium. falciparum. Antibodies raised against synthetic peptides and recombinantly produced constructs from the unique repeating sequence of the P. falciparum CS protein have been shown to possess biologic activities possibly predictive of protection against sporozoite challenge, i.e. they mediate the circumsporozoite precipitation reaction and block sporozoite invasion of hepatocytes in vitro. These studies have led to the development of vaccines designed to protect against infection with the sporozoite stage of P. falciparum which has been tested in a Phase I safety and immunogenicity trial and a small Phase IIA efficacy trial in volunteers in the United States. Although this initial vaccine was not sufficiently immunogenic to justify Phase IIb trials, additional vaccines using improved adjuvants are now under development by WRAIR and others. Planning for a Phase IIb trial will

require accurate information on the expected transmission of falciparum malaria at the study site and information regarding the logistical, technical, and community support for conducting such a trial.

Kenya has been chosen by WRAIR as the primary candidate site for the first Phase IIb trial. Studies must now provide that information that will enable accurate estimation of the expected P.falciparum attack rate in volunteers staying in these areas for 2-8 weeks and the optimal time to conduct such a trial. Since volunteers will receive their last booster dose of vaccine one month before the study begins, data must be collected which will enable the investigators confidently to predict peak transmission one month in advance of its onset. This proposal details the epidemiologic and entomologic approach that will be used to gather these data.

In hyper- and holoendemic malarious areas the prevalence of malaria infection is highest in children less than 6 years of age, who presumably have not yet developed significant protective immunity against malaria. These young children most closely resemble non-immune adults in regard to their susceptibility to malaria. Determination of the attack rate of falciparum malaria in these children is crucial to confident prediction of the attack rate in non-immune volunteers, a factor critical to the determination of the sample size required to predict the protective efficacy of the vaccine. The previous grant determined these attack rates over a period of approximately two years. While the determination of the entomologic inoculation rate (man biting rate x sporozoite rate) and incidence of malaria in human populations form the core of these data, the ability to predict peak transmission is dependent on continued entomologic and meteorologic studies that will help insure an optimal Phase IIb trial of a vaccine.

Saradidi, Siaya District, near Kisumu in Nyanza Province, Kenya, has been chosen as the principal study site because it has met the following criteria:

1. High prevalence of falciparum malaria with low prevalence of other malaria species.

2. Large data base collected by Kenyan and CDC investigators during the past 7 years.

3. Demonstrated community support for malaria studies

4. Favorable logistics.

5. A low prevalence of other diseases and an absence of filariasis and leishmaniasis which volunteers would be at risk of contracting.

A second site has been selected adjacent to the new KEMRI laboratory 10 km west of Kisumu. Similar studies to those described for Saradidi will be carried out at this site.

Studies in cell-mediated immunity and the role of cytokines in the development of resistance to malaria are now necessary in support of vaccine development efforts at WRAIR. Data generated in collaboration with WRAIR and others during the previous grant period supports the necessity for antigenic epitopes in a malaria vaccine which will stimulate cellular as well as humoral immunity. Individuals in the study areas show varying degrees of resistance to malaria; it is important to characterize the immunological status of these individuals in relation to their malarial resistance.

Malaria Abstracts

SAFETY AND IMMUNOGENICITY OF A RECOMBINANT FALCIPARUM SPOROZOITE VACCINE WITH PRIOR EXPOSURE TO NATURAL SPOROZOITES IN KENYA.

J.A. Sherwood, C.N. Oster, M. Adoyo-Adoyo, J.C. Beier, G.S. Gachihi, P.M. Nyakundi, J. Koros, W.R. Ballou, A.D. Brandling-Bennett, I.K. Schwartz, J.B.O. Were, C.R. Roberts, J.F. Young, P. Reeve and J.D. Chulay.

Fifteen normal volunteers, with prior exposure to natural falciparum sporozoites, were vaccinated once with 10 to 800 microgram doses of the recombinant falciparum sporozoite vaccine FSV-1, (R32tet32) containing the tetrapeptides repeats [(asn-ala-asn-pro)15-(asn-val-asp-pro)1]2. Ten volunteers were vaccinated with placebo. With vaccine and placebo respectively, minimal transient local inflammation occurred in 3 and 3, and transient headache in 2 and 3. No adverse effects were detected by laboratory tests. Specific antibodies to R32tet32 were assayed by ELISA with 1:100 dilutions of serum. Doses of 10, 30 and 100 micrograms gave no significant rise in antibody. Among the 10 volunteers receiving 300 and 800 microgram doses, prior to vaccination, 4 had less than 0.2 and 6 had 0.4 to 0.9 optical density units. with the 300 microgram dose, levels increased in 1 of 3 receiving vaccine, from 0.15 to 0.34. With the 800 microgram dose, levels increased in 2 of the 3 receiving vaccine, from 0.62 to 1.6 and from 0.36 to 1.0 units. The highest levels were similar to those obtained in vaccine volunteers without prior exposure to natural sporozoites and to naturally acquired, apparently non-protective, antisporozoite antibody. Antibody levels fell after 1 to 2 months following vaccination. This recombinant sporozoite vaccine appears safe, but insufficiently immunogenic, in those with prior naturally acquired antisporozoite antibody, to produce antibody higher than apparently nonprotective natural or induced levels. This vaccine was not able to induce long lasting antibody levels even in those with prior exposure to natural sporozoites. consideration should be given to an improved recombinant vaccine with T-cell receptor sites capable of inducing longer lasting antibody levels.

MALARIA SPOROZOITE DETECTION BY DISSECTION AND ELISA TO ASSESS INFECTIVITY OF AFROTROPICAL *ANOPHELES* (DIPTERA: CULICIDAE)

JOHN C. BEIER, PETER V. PERKINS, JOSEPH K. KOROS, FRED K. ONYANGO, THOMAS P. GARGAN, ROBERT A. WIRTZ, DAVY K. KOECH, AND CLIFFORD R. ROBERTS

J. Med.Entomol.27(3):377-384(1990)

Malaria infection rates determined by dissection and *Plasmodium falciparum* enzyme-linked immunosorbent assay (ELISA) were compared for 26,935 *Anopheles gambiae* Giles sensu lato and 17,739 *Anopheles funestus* Giles collected during 20 mo in western Kenya. ELISA infection rates were about 43% higher than dissection sporozoite rates. In dissection-negative *Anopheles*, circumsporozoite (CS) protein was detected by ELISA in 5.2% of 10,017 salivary gland samples and in 12.2% of 237 thorax samples. The accuracy of dissection and ELISA techniques was compared by the following tests on a group of 352 field-collected *Anopheles* (held 10 d to ensure sporogonic development): salivary gland dissection, examination of Giemsa-stained dissection slides, ELISA tests on salivary gland and thorax body parts, and microscopic techniques for determining sporozoite loads. Respective infection rates were 9.9%, 10.8%, and 15.6% for dissection, stained slides, and ELISA. Sporozoite loads were associated significantly with ELISA absorbance values ($r = 0.76$). Compared with Giemsa-stained dissection slide results, the sensitivity of sporozoite detection was 92.1% for dissection compared with 78.9% for ELISA; specificity was 100.0% for dissection versus 92.0% for ELISA. Immunological detection of CS protein in head-thorax samples of Afrotropical vectors overestimated the proportion of infective *Anopheles* because the comparison of techniques indicated that 45.4% of the ELISA positive *Anopheles* did not contain salivary gland sporozoites.

PLASMODIUM SPECIES IDENTIFICATION BY ELISA FORSPOROZOITES REMOVED FROM DRIED DISSECTION SLIDES

JOHN C. BEIER, ROBERT S. COPELAND, FRED K. ONYANGO, CHARLES M. ASIAGO, MUTALIB RAMADHAN, DAVY K. KOECH & CLIFFORD R. ROBERTS

The feasibility of allowing sporozoites to dry on dissection slides before Plasmodium species identification by ELISA was determined for salivary gland sporozoites from Anopheles gambiae s.l. and An. funestus collected in western Kenya. In 119 gland infections containing a geometric mean of 1,222 sporozoites, a mean of 72.5% of sporozoites were removed in 60 µl saline from slides at the time of dissection. Each of the 119 samples was divided into three 18 µl aliquots. Subsamples were either stored at -70°C, dried in vials, or dried on a microslide. When tested by Plasmodium falciparum ELISA, positive reactions were observed for 86.6% of frozen samples, 70.6% of samples held dry in vials and 50.4% of samples held dry on microslides for one month. For 90 gland infections where coverslips were removed and slides were left to dry for one month before adding blocking buffer, 81.1% were positive for P. falciparum. This was not significantly different from either frozen gland samples where 85.5% of 392 infections were identified or frozen gland plus corresponding thorax samples where 86.2% of 160 samples were identified. In malaria field studies, where it is not always practical to freeze samples, sporozoites from dissected mosquitoes can be preserved adequately for ELISA identification by simply removing c~overslips and drying dissection slides.

QUANTITATION OF MALARIA SPOROZOITES TRANSMITTED IN VITRO DURING SALIVATION BY WILD AFROTROPICAL ANOPHELES

JOHN C. BEIER, FRED K. ONYANGO, JOSEPH K. KOROS, MUTALIB RAMADHAN, ROSE OGWANG, ROBERT A. WIRTZ, DAVY K. KOECH and CLIFFORD R. ROBERTS

The malaria transmission potential of wild, infected Anopheles from western Kenya was evaluated by determining the number of sporozoites transmitted in vitro by salivation when their mouthparts were placed into capillary tubes containing either sucrose or blood. With sucrose, 86.6% of

102 infected Anopheles transmitted a geometric mean (GM) of 3.84 sporozoites (range: 1-34). With blood, 23.1% of 104 infected Anopheles, tested on the day of collection, transmitted a GM of 2.30 sporozoites (range 1-117). For Anopheles held 5 days postcapture before testing with blood, 53.6% of 56 transmitted a GM of 6.04 sporozoites (range: 1-420). Transmitting Anopheles contained significantly more salivary gland sporozoites than non-transmitters. No significant differences in transmission potential were detected between Anopheles gambiae Giles sensu lato and Anopheles funestus Giles. Sporozoites were detected microscopically in the salivary duct from heads in 80.3% of 117 infected Anopheles (GM-11.2, range:1-71). Sporozoite detection in mosquito heads by ELISA was 25% less efficient than microscopic detection. Over 98% of the infective Anopheles transmitted less than 25 sporozoites and transmission efficiency was negatively correlated with the sporozoite load. Transmitted sporozoites represented only about 3% of the total sporozoites in the salivary glands suggesting that sporozoite transmission may be restricted to only sporozoites in the salivary duct at the time of feeding. Results are discussed in relation to sporozoite vaccine development.

RELATIVE ABUNDANCE AND BLOOD FEEDING BEHAVIOR OF NOCTURNALLY ACTIVE CULICINE MOSQUITOES IN WESTERN KENYA

John C. Beier, Walter O. Odago, Fred K. Onyango, Charles M. Asiago, Davy K. Koech and Clifford R. Roberts

Culicine species composition, relative abundance and degree of contact with man were studied for 1 year at 2 sites in western Kenya. In Kisian, a site on the shores of Lake Victoria containing extensive papyrus swamp, collections included 20,910 adult female representing 19 species. Species diversity was 3 times lower in Saradidi, a site with an ecotype of savanna vegetation; 4,312 mosquitoes of 11 species were collected. Common species in Kisian included Culex quinquefasciatus (71.4%), Mansonia uniformis (15.8%), Ma. africana (6.2%), Aedes mcintoshi (2.0%), Coquillettidia fuscopennata (1.9) and Ae.ochraceus (1.8%). Common species in Saradidi included Cx. quinquefasciatus (92.7%), Cx. nebulosus (4.5%) and Ma. uniformis (1.0%). Human-bait collections identified 16 man-biting culicine species in Kisian and 9 in Saradidi; human-bait collections more effectively detected culicine species than 4 other techniques. Biting rates in Kisian for

the 5 most common species were 1.8, 14.6 and 13.5 times higher than in Saradidi for indoor, outdoor and tent collections, respectively. Resident exposure indoors to nocturnally-biting culicine mosquitoes was estimated at 1,277 bites/man/year in Kisian and 720 in Saradidi. Bloodmeal identification for 1,083 mosquitoes confirmed that the common culicine species feed primarily on humans and cows. This study provides baseline information to assess potential risks of mosquito-borne pathogen transmission at 2 sites targeted for future malaria vaccine trials.

ANOPHELES GAMBIAE COMPEX EGG-STAGE SURVIVAL IN DRY SOIL FROM LARVAL DEVELOPMENT SITES IN WESTERN KENYA

John C. Beier,l Robert Copeland, Christopher Oyaro, Alex Masinya, Walter 0. Odago, Samuel Oduor, Davy K. Koech and Clifford R. Roberts

The potential for Anopheles egg survival in dry soil from larval development site was investigated in Kisian, a rural malaria-endemic village in western Xenya. A total of 230 dry soil samples collected in 1987, 1988 and 1989 yielded 126 firstinstar Anopheles gambiae sensu lato larvae after flooding with water. These larvae were from dried animal (cow and hippo) hoofprints along streams (57.9%), from dried edges of permanent and temporary pools (41.3%), and from dried stream beds (0.8%). Larval density was 1.2 larvae per kg of soil from positive microhabitats in 1987 and 2.4 larvae per kg in 1988. Firstinstar larvae were observed from days 2 to 5 after soil samples were flooded. Thirteen larvae from the 1989 soil samples, reared to adults, were identified by DNA probes as Anopheles gambiae sensu strictu (N=6) and Anopheles arabiensis (N=7). Under three experimental conditions, eggs from field-collected females remained viable for as long as 12 days for A. gambiae s.l. and 10 days for An. funestus. In western Kenya, the adaptation of two species in the An. gambiae complex to remain viable in dry soil may represent a significant, short-term population survival mechanism.

EFFECT OF PLASMODIUM FALCIPARUM ON THE FEEDING BEHAVIOR OF NATURALLY INFECTED ANOPHELES MOSQUITOS IN WESTERN KENYA

Wekesa, Joseph W., Copeland, Robert S., and Mwangi, Richard W.

Feeding behavior was compared between infected and uninfected, field-collected groups of Anopheles gambiae sensu lato and Anopheles funestus from western Kenya. A significantly greater pe centage (81%) of Plasmodium falciparum-infected A. gambiae s.l. females probed on experimental hosts (hamsters) than did uninfected females (38(%). Among those females which initiated probing, there was no effect of infection status on the ability to take a bloodmeal. P. falciparum-infected A. gambiae s.l. probed more often (mean=4.0) and for a longer time (mean=277 sec) than did their uninfected counterparts (mean 2.4 probes and 214 sec probing time). Results for the small number of A. funestus which fed followed the same trend. Among infected A. gambiae s.l. females, there was no effect of sporozoite density on either number of probes made or total probing time. Among uninfected females, there was no difference in feeding behavior between nulliparous and parous females. In laboratory experiments, female age had no effect on blood-feeding behavior. Our findings provide evidence that natural malaria infection modifies feeding behaviour of Anopheles females.

Leishmaniasis:

Diagnosis, treatment, and prevention of visceral and cutaneous leishmaniasis continue to be inadequate for ready application to field medical facilities. However, the presence of several endemic foci and a leishmaniasis clinical research facility make Kenya exceptionally capable of research relevant to exposed military populations.

Leishmaniasis Abstracts

PHLEBOTOMUS GUGGISBERGI (DIPTERA: PSYCHODIDAE), A VECTOR OF *LEISHMANIA TROPICA* IN KENYA

PHILLIP G. LAWYER, YEMANE B. MEBRAHTU, PHILIP M. NGUMBI, PANWEL MWANYUMBA, JOHN MBUGUA, GABRIEL KIILU, DAVID KIPKOECH, JOSEPH NZOVU, AND CHRISTOPHER O. ANJILI

Sand flies were collected in light traps and on oiled papers at 4 active case sites of human cutaneous leishmaniasis due to Leishmania tropica at Muruku Sublocation, Laikipia District, Kenya. Nearly 5,200 females 6 species, including 2 Phlebotomus species (P. guggisbergi and P. duboscqi), were dissected and examined for flagellates. Of 3,867 P. guggisbergi females collected at a multiple case site, 167 (4.3%) harbored mature leishmanial infections, to include metacyclic promastigotes, while all other flies were negative. Of the infected flies, 164 were collected in a cave near the patients' home, 2 from crevices on the escarpment immediately behind the house, and 1 from the bedroom of one of the patients. One hundred sixty-four of the isolates were successfully grown in Schneider's Drosophila medium and harvested for- typing by cellulose-acetate electrophoresis. Isoenzyme profiles of the first 22 of these were compared with those of WHO reference strains and well characterized local strains using 12 enzyme loci. The isolates yielded isoenzyme migration patterns that were indistinguishable from those of 2 L. tropica reference strains and of 6 L. tropica patient isolates from the same locality. This is the first reported isolation of Leishmania parasites from P. guggisbergi, the first reported isolation of L. tropica from a sand fly in Kenya, and the first confirmed isolation of this Leishmania from a sand fly other than P. sergenti. The finding of such a large number of P. guggisbergi naturally harboring mature infections of L. tropica at an active

case site of cutaneous leishmaniasis due to this agent strongly implicates this fly as a vector.

DEVELOPMENT OF *LEISHMANIA MAJOR* IN PHLEBOTOMUS DUBOSCQI AND SERGENTOMYIA SCHWETZI (DIPTERA: PSYCHODIDAE)

PHILLIP G. LAWYER, PHILIP M. NGUMBI, CHRISTOPHER O. ANJILI, SHADRACK O. ODONGO, YEMANE B. MEBRAHTU, JOHN 1. GITHURE, DAVY K. KOECH, AND CLIFFORD R. ROBERTS

The extrinsic development of *Leishmania major* was observed in 2 manbiting sand flies, *Phlebotomus duboscqi*, a known vector, and *Sergentomyia schwetzi*, an assumed non-vector. Flies fed on a leishmanial lesion on the nose of a hamster were examined for infection at 0, 6, 12, 18, 24, 36, 48, and 60 hr and at ~24 hr intervals from day 3 to day 14 post-feeding. Infection rates, determined by light microscopy, were 47% (n = 258) in P. *duboscqi* and 5% (n = 162) in S. *schwetzi*. Transformation from amastigotes to "procyclic" promastigotes occurred in both species at 6-18 hr post-feeding. In P. *duboscqi*, the parasites multiplied rapidly and developed through as many as 10 forms, including at least 3 dividing-promastigote forms. Metacyclic promastigotes, the "infective" form, appeared at 6 days post-feeding, first in the region of the stomodeal valve, then in the pharynx, cibarium, and proboscis. In a single attempt 14 days post-feeding, a P. *duboscqi* transmitted *L. major* to a mouse by bite. In contrast, the parasites multiplied slowly in S. *schwetzi*, and did not develop beyond "procyclic" promastigotes. The parasites did not migrate anteriorly nor survive beyond 90 hr post-feeding, indicating that S. *schwetzi* is not a vector of *L. major*. Classical strategies for vector incrimination may be confounded by the isolation of non-infective early developmental forms of *Leishmania* from wild-caught non-vectors.

LEISHMANIA INFANTUM SENSU LATO ISOLATED FROM A GIANT RAT (*CRICETOMYS GAMBIANUS*) CAPTURED AT A CASE SITE OF HUMAN CUTANEOUS LEISHMANIASIS IN KENYA

PHILLIP G. LAWYER, PHILIP M. NGUMBI, CHRISTOPHER O. ANJILI, SHADRACK O. ODONGO, YEMANE B. MEBRAHTU, JOHN I. GITHURE, DAVY K. KOECH, AND CLIFFORD R. ROBERTS

Investigations were conducted at a multiple-case site within a recently discovered rural focus of human cutaneous leishmaniasis. Six cutaneous *Leishmania* isolates previously aspirated from patients at this and neighboring case sites were cultured and subsequently typed by cellulose acetate electrophoresis as *L. tropica*. An isolate from 1 of 6 giant rats *(Cricetomys gambianus)* captured near the patients' house was grown on NNN overlayed with Schneider's *Drosophila* medium. Isoenzyme profiles of this isolate, of *L. tropica* patient isolates, and WHO reference strains were compared by cellulose acetate electrophoresis using 12 enzyme loci (Mdh, Me, Icd, 6Pgd, G6pd, Nh1, Nh2, Nh3, Mpi, Gpi, Pgm1, and Pgm2). Unexpectedly, the isolate from the giant rat was found to be indistinguishable from an *L. infantum* reference strain (MHOM/TN/80/LEM235, NLB-317) for all enzymes tested. This is the first isolation of *L. infantum* from any host in Kenya. A preliminary survey for potential vector sand flies in the immediate vicinity of the house and in a nearby cave produced several sand fly species including *Phlebotomus guggisbergi* and *P.duboscqi*. Either of these fly species could be involved in the transmission of cutaneous leishmaniasis caused by *L. infantum* and/or *L. tropica*.

CONCURRENT INFECTION WITH *LEISHMANIA DONOVANI* AND *LEISHMANIA MAJOR* IN A KENYAN PATIENT: CLINICAL DESCRIPTION AND PARASITE CHARACTERIZATIONS.

L. Hendricks, Y. Mebrahtu, P. Lawyer, R. Muigai, C. Oster, P. Perkins, D. Koech, H. Pamba, and C. Roberts. Kenya Medical Research Institute, U.S.Army Medical Research Unit, and University of Nairobi Medical Faculty, Nairobi, Kenya.

An indigenous adult male from Baringo District, Kenya developed kala-azar. His village lies within foci of kala-azar and cutaneous leishmaniasis due to *L. donovani* and *L. major*. A splenic aspirate grew *Leishmania* promastigotes and the patient was treated for kala-azar and discharged. Four months later, after relapse, he returned for a second course of treatment. During this second visit the patient was among a group of randomly sampled participants in a study of diagnosis of leishmaniasis using urine and nasopharyngeal cultures. Urine, nasopharyngeal, and splenic samples were found positive for *Leishmania*. Splenic and urine cultures were cryopreserved. Cellulose acetate electrophoresis using 17 enzymes (LDH, MDH, ME, ICD, 6PGD, GAPDH1, G6PD, HK PFK, AK, ACP, NH1, NH2, MPI, GPI, PGM1 and PGM2) revealed a mixed infection with *L. donovani and L. major*. The original isolate from the splenic aspirate was then characterized and showed mixed infection. Stationary-phase promastigotes from these cultures were inoculated subcutaneously, intraperitoneally, and intracardially into Syrian hamsters and Balb/c mice. Six and a half months later two hamsters and two mice developed both kala-azar and cutaneous leishmaniasis. Parasites from liver, spleen, bone marrow, lymph node, and skin were identified by CAE as *L. donovani* and/or *L. major*.

EXPERIMENTAL TRANSMISSION OF *LEISHMANIA MAJOR* TO VERVET MONKEYS *(CERCOPITHECUS AETHLOPS)* BY BITES OF *PHLEBOTOMUS DUBOSCQI* (DIPTERA: PSYCHODIDAE)

P.G. Lawyer, J.I. GITHURE, C.O. ANJILI, J.O. Olobo, D.K. Koech, & G.D.F. Reid

Experimental transmission of *Leishmania major to* vervet monkeys *(Cercopithecus aethiops) was* accomplished by bites of *Phlebotomus duboscqi*

sandflies. Three-day-old, laboratory-reared P. *duboscqi* were fed from leishmanial lesions on hamsters infected with *L. major*. The flies were refed on monkeys at 10 days postinfection. Five adult male vervet monkeys were used in concurrent transmission trials. Two of the monkeys received subcutaneous inoculations with stationary-phase promastigotes (2x106 promastigotes in 0.1 ml of medium) on the base of the tail. Putatively infected P. *duboscqi* were allowed to feed on the remaining 3 monkeys at sites on the base of the tail and on the right eyebrow. Challenges by sandfly bites resulted in multiple leishmanial lesions at all bite sites and, consequently, more lesion area than produced by needle challenges. Results of postfeeding dissections of sandflies indicate that multiple lesions can be caused by bites of a single fly, and that probing alone, without imbibing blood, is sufficient for transmission. These first experimental transmissions of *L. major* to vervets by bites of P. *duboscqi* demonstrate that sandfly challenge is an efficient alternative to needle challenge, making available a unique Leishmania-sandfly-nonhuman primate model for use in vaccine development.

VISCERAL LEISHMANIASIS UNRESPONSIVE TO PENTOSTAM CAUSED BY *LEISHMANIA TROPICA* IN KENYA

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We report the characterization of 6 *Leishmania tropica* isolates from 2 patients with visceral leishmaniasis who were unresponsive to treatment with sodium stibogluconate. The *Leishmania* isolates, MHOM/KE/81/NLB-029A, -029XIB, and -029XIC and MHOM/KE/81/NLB-030I, -030B, and -030XXA, all from splenic aspirates, were characterized by cellulose acetate electrophoresis using 11 enzymes: malate dehydrogenase, malic enzyme, phosphogluconate dehydrogenase, glucose-6-phosphate dehydrogenase, superoxide dismutase, glutamate-oxaloacetate transaminase, adenylate kinase, nucleoside hydrolase, mannose phosphate isomerase, glucose phosphate isomerase, and phosphoglucomutase. Isozyme migration patterns were indistinguishable from those of 2 WHO reference strains of *Leishmania tropica* (MHOM/SU/60/LRC-L39, NLB-305 and MHOM/IQ/OO/LRC-L36, NLB-067). These are the first reported cases of visceral

leishmaniasis (kala-azar) caused by *L. tropica* in Africa; these cases were refractory to sodium stibogluconate.